

## MINIREVIEW

# Updated Functional Classification of $\beta$ -Lactamases<sup>▽</sup>

Karen Bush<sup>1\*</sup> and George A. Jacoby<sup>2</sup>

*Department of Biology, Indiana University, Bloomington, Indiana,<sup>1</sup> and Lahey Clinic, Burlington, Massachusetts<sup>2</sup>*

Two classification schemes for  $\beta$ -lactamases are currently in use. The molecular classification is based on the amino acid sequence and divides  $\beta$ -lactamases into class A, C, and D enzymes which utilize serine for  $\beta$ -lactam hydrolysis and class B metalloenzymes which require divalent zinc ions for substrate hydrolysis. The functional classification scheme updated herein is based on the 1995 proposal by Bush et al. (K. Bush, G. A. Jacoby, and A. A. Medeiros, *Antimicrob. Agents Chemother.* 39:1211–1233, 1995). It takes into account substrate and inhibitor profiles in an attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates. Major groupings generally correlate with the more broadly based molecular classification. The updated system includes group 1 (class C) cephalosporinases; group 2 (classes A and D) broad-spectrum, inhibitor-resistant, and extended-spectrum  $\beta$ -lactamases and serine carbapenemases; and group 3 metallo- $\beta$ -lactamases. Several new subgroups of each of the major groups are described, based on specific attributes of individual enzymes. A list of attributes is also suggested for the description of a new  $\beta$ -lactamase, including the requisite microbiological properties, substrate and inhibitor profiles, and molecular sequence data that provide an adequate characterization for a new  $\beta$ -lactam-hydrolyzing enzyme.

Hydrolysis of  $\beta$ -lactam antibiotics by  $\beta$ -lactamases is the most common mechanism of resistance for this class of antibacterial agents in clinically important Gram-negative bacteria. Because penicillins, cephalosporins, and carbapenems are included in the preferred treatment regimens for many infectious diseases, the presence and characteristics of these enzymes play a critical role in the selection of appropriate therapy.

$\beta$ -Lactamase production is most frequently suspected in a Gram-negative bacterial isolate that demonstrates resistance to a  $\beta$ -lactam antibiotic. Due to more sophisticated molecular approaches than were previously available, it has become increasingly easy to obtain nucleotide sequences, with their deduced amino acid sequences, for the genes encoding these enzymes in  $\beta$ -lactam-resistant clinical isolates. By late 2009, the number of unique protein sequences for  $\beta$ -lactamases exceeded 890 (16; G. Jacoby and K. Bush, <http://www.lahey.org/Studies/> [a site that contains additional literature and GenBank accession number references for  $\beta$ -lactamases in various functional groups]). Thus, it is important that a systematic process be established for tracking these enzymes.

Classification of  $\beta$ -lactamases has traditionally been based on either the functional characteristics of the enzymes (16, 55) or their primary structure (2). The simplest classification is by protein sequence, whereby the  $\beta$ -lactamases are classified into four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs (2, 3, 29, 46). Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl enzyme through an active site serine, whereas class B  $\beta$ -lactamases are metalloenzymes that utilize at least one

active-site zinc ion to facilitate  $\beta$ -lactam hydrolysis. Although a structural approach is the easiest and least controversial way to classify such a diverse set of enzymes, a functional classification provides the opportunity to relate these varied enzymes to their clinical role, i.e., by providing selective resistance to different classes of  $\beta$ -lactam antibiotics. Functional groupings, admittedly, can be more subjective than structural classes, but they aid the clinician and laboratory microbiologist in correlating the properties of a specific enzyme with the observed microbiological resistance profile for a clinical isolate. Historically, functionality has been the overriding consideration in defining the role of a particular  $\beta$ -lactamase in the medical setting (55). Thus, it seems appropriate to continue to group these diverse enzymes according to their hydrolytic and inhibition properties.

### UPDATED FUNCTIONAL CLASSIFICATION

Table 1 depicts an expanded version of the functional classification scheme proposed initially by Bush in 1989 (13) and expanded in 1995 (16). This table aligns structural and functional classifications as closely as possible, based on the available information in the public domain. New functional subgroups have been added to the scheme as a result of identification and expansion of major  $\beta$ -lactamase families in which variants continue to be identified on a regular basis (Table 2). As in the earlier functional classifications, enzymes were aligned based on their ability to hydrolyze specific  $\beta$ -lactam classes and on the inactivation properties of the  $\beta$ -lactamase inhibitors clavulanic acid, sulbactam, and tazobactam. A description of each of the functional groups follows.

**Group 1 cephalosporinases.** Group 1 enzymes are cephalosporinases belonging to molecular class C that are encoded on the chromosomes of many *Enterobacteriaceae* and a few other

\* Corresponding author. Mailing address: Department of Biology, Indiana University, Bloomington, IN 47401. Phone: (812) 855-1542. Fax: (812) 333-6192. E-mail: karbush@indiana.edu.

<sup>▽</sup> Published ahead of print on 7 December 2009.

TABLE 1. Classification schemes for bacterial  $\beta$ -lactamases, expanded from Bush et al. (16)

Bush-Jacoby group (2009)	Bush-Jacoby-Medeiros group (1995)	Molecular class (subclass)	Distinctive substrate(s)	Inhibited by		Defining characteristic(s)	Representative enzyme(s)
				CA or TZB <sup>a</sup>	EDTA		
1	1	C	Cephalosporins	No	No	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	<i>E. coli</i> AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	NI <sup>b</sup>	C	Cephalosporins	No	No	Increased hydrolysis of ceftazidime and often other oxymino- $\beta$ -lactams	GC1, CMY-37
2a	2a	A	Penicillins	Yes	No	Greater hydrolysis of benzylpenicillin than cephalosporins	PC1
2b	2b	A	Penicillins, early cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin and cephalosporins	TEM-1, TEM-2, SHV-1
2be	2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	Increased hydrolysis of oxymino- $\beta$ -lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
2br	2br	A	Penicillins	No	No	Resistance to clavulanic acid, sulbactam, and tazobactam	TEM-30, SHV-10
2ber	NI	A	Extended-spectrum cephalosporins, monobactams	No	No	Increased hydrolysis of oxymino- $\beta$ -lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam	TEM-50
2c	2c	A	Carbenicillin	Yes	No	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
2ce	NI	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and ceftiofame	RTG-4
2d	2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
2de	NI	D	Extended-spectrum cephalosporins	Variable	No	Hydrolyzes cloxacillin or oxacillin and oxymino- $\beta$ -lactams	OXA-11, OXA-15
2df	NI	D	Carbapenems	Variable	No	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48
2e	2e	A	Extended-spectrum cephalosporins	Yes	No	Hydrolyzes cephalosporins. Inhibited by clavulanic acid but not aztreonam	CepA
2f	2f	A	Carbapenems	Variable	No	Increased hydrolysis of carbapenems, oxymino- $\beta$ -lactams, cephamycins	KPC-2, IMI-1, SME-1
3a	3	B (B1)	Carbapenems	No	Yes	Broad-spectrum hydrolysis including carbapenems but not monobactams	IMP-1, VIM-1, CcrA, IND-1
		B (B3)					L1, CAU-1, GOB-1, FEZ-1
3b	3	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems	CphA, Sfh-1
NI	4	Unknown					

<sup>a</sup> CA, clavulanic acid; TZB, tazobactam.<sup>b</sup> NI, not included.

organisms (27). They are more active on cephalosporins than benzylpenicillin and are usually resistant to inhibition by clavulanic acid and active on cephamycins, such as cefoxitin. They have a high affinity for aztreonam ( $K_i$  values as low as 1 to 2 nM), in contrast to the class A cephalosporinases (14, 15). A few have unusual properties, such as a lack of activity on cefoxitin (6), inhibition by clavulanate or tazobactam (5, 69), or production of resistance to cefotaxime but not ceftazidime (73). In many organisms, including *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, AmpC expression is low but inducible on exposure to certain  $\beta$ -lactams, such as amoxicillin, ampicillin, imipenem, and clavulanic acid (17, 27, 34, 67). In other organisms, including *Acinetobacter baumannii* and *Escherichia coli*, one or more components of the induction system are missing. When produced in large amounts, especially in a host with reduced  $\beta$ -lactam accumulation, group 1 enzymes can provide resistance to carbapenems, especially ertapenem (11, 28, 51). Plasmid-mediated group 1 enzymes in the CMY, ACT, DHA,

FOX, MIR, and other families have been known since 1989 but are currently less common than plasmid-mediated subgroup 2be extended-spectrum  $\beta$ -lactamases (ESBLs) (27).

The new subgroup 1e enzymes are group 1 variants with greater activity against ceftazidime and other oxymino- $\beta$ -lactams as a result of amino acid substitutions, insertions, or deletions (44). They have been termed extended-spectrum AmpC (ESAC)  $\beta$ -lactamases and include GC1 in *E. cloacae* (45) and plasmid-mediated CMY-10 (33), CMY-19 (64), CMY-37 (1), and others (21). An AmpC variant from *P. aeruginosa* with increased activity against imipenem has also been recently described (57). Clinically significant resistance is most often conferred when the producing organism also has a porin mutation (36).

**Group 2 serine  $\beta$ -lactamases.** Functional group 2  $\beta$ -lactamases, including molecular classes A and D, represent the largest group of  $\beta$ -lactamases, due primarily to the increasing identification of ESBLs during the past 20 years (Fig. 1). Subgroup 2a penicillinases represent a small group of  $\beta$ -lactamases

TABLE 2. Major families of  $\beta$ -lactamases of clinical importance

Enzyme family <sup>a</sup>	Functional group or subgroup	No. of enzymes <sup>b,c</sup>	Representative enzymes
CMY	1, 1e	50	CMY-1 to CMY-50
TEM	2b, 2be, 2br, 2ber	172	
	2b	12	TEM-1, TEM-2, TEM-13
	2be	79	TEM-3, TEM-10, TEM-26
	2br	36	TEM-30 (IRT-2), TEM-31 (IRT-1), TEM-163
	2ber	9	TEM-50 (CMT-1), TEM-158 (CMT-9)
SHV	2b, 2be, 2br	127	
	2b	30	SHV-1, SHV-11, SHV-89
	2be	37	SHV-2, SHV-3, SHV-115
	2br	5	SHV-10, SHV-72
CTX-M	2be	90	CTX-M-1, CTX-M-44 (Toho-1) to CTX-M-92
PER	2be	5	PER-1 to PER-5
VEB	2be	7	VEB-1 to VEB-7
GES	2f	15 <sup>d</sup>	GES-2 to GES-7 (IBC-1) to GES-15
KPC	2f	9	KPC-2 to KPC-10
SME	2f	3	SME-1, SME-2, SME-3
OXA	2d, 2de, 2df	158	
	2d	5	OXA-1, OXA-2, OXA-10
	2de	9	OXA-11, OXA-14, OXA-15
	2df	48 <sup>e</sup>	OXA-23 (ARI-1), OXA-51, OXA-58
IMP	3a	26	IMP-1 to IMP-26
VIM	3a	23	VIM-1 to VIM-23
IND	3a	8	IND-1, IND-2, IND-2a, IND-3 to IND-7

<sup>a</sup> Enzyme families include those for which numbers have been assigned based on primary amino acid structures (G. Jacoby and K. Bush, <http://www.lahey.org/Studies/>).

<sup>b</sup> Compiled through December 2009.

<sup>c</sup> The sum of the subgroups in each family does not always equal the total number of enzymes in each family, because some enzyme numbers have been withdrawn, and some enzymes have not been assigned a functional designation by the investigators who provided the amino acid sequence.

<sup>d</sup> GES-1, unlike other members of the GES family, has little detectable interaction with imipenem (49).

<sup>e</sup> Nine clusters of OXA carbapenemases with their individual members have been designated in Table 6 in reference 52.

with a relatively limited spectrum of hydrolytic activity and are the predominant  $\beta$ -lactamases in Gram-positive cocci, including the staphylococci (30) and occasionally enterococci (74). These enzymes preferentially hydrolyze benzylpenicillin and

many penicillin derivatives, with poor hydrolysis of cephalosporins, carbapenems, or monobactams at rates usually  $\leq 10\%$  those for benzylpenicillin or ampicillin. An exception is the facile hydrolysis of nitrocefin by the subgroup 2a enzymes.

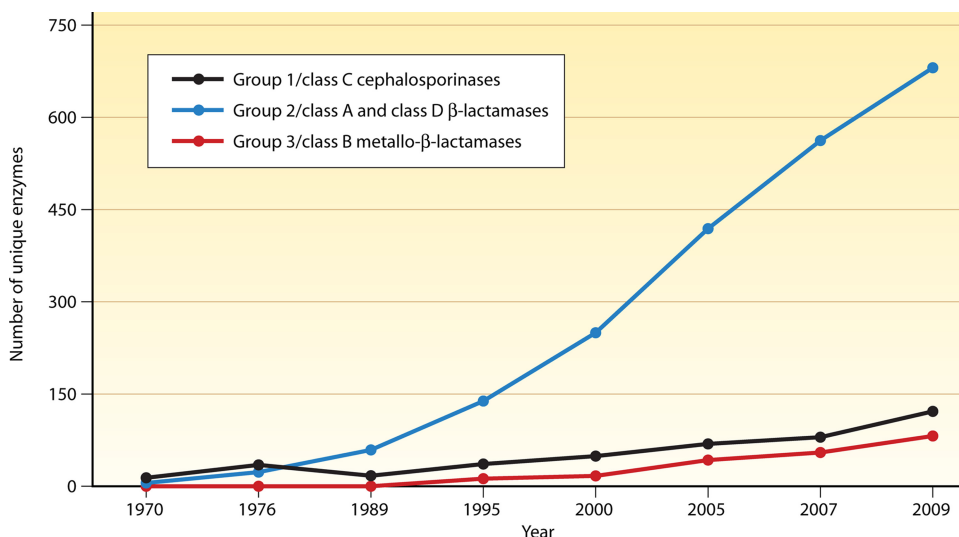


FIG. 1. Increase in numbers of group 1, 2, and 3  $\beta$ -lactamases from 1970 to 2009. Shown are group 1/class C cephalosporinases (black), group 2/class A and class D  $\beta$ -lactamases (blue), and group 3/class B metallo- $\beta$ -lactamases (red).

Subgroup 2a  $\beta$ -lactamases are inhibited by clavulanic acid and tazobactam with 50% inhibitory concentrations ( $IC_{50}$ s) of usually  $<1 \mu\text{M}$ , assuming at least 5 min of preincubation of enzyme and inhibitor. The majority of these enzymes are chromosomal, although some staphylococcal penicillinases are plasmid encoded. This subgroup, which numbered 20 in 1995, has increased to only 25 in 2009. This may be because a true penicillinase does not cause significant clinical resistance for those  $\beta$ -lactams in predominant current use.

Subgroup 2b  $\beta$ -lactamases readily hydrolyze penicillins and early cephalosporins, such as cephaloridine and cephalothin, and are strongly inhibited by clavulanic acid and tazobactam. They include the TEM-1, TEM-2, and SHV-1 enzymes, the most common plasmid-mediated  $\beta$ -lactamases identified in the 1970s and early 1980s (38, 58). Since the 1995  $\beta$ -lactamase compilation (16), at least 9 TEM and 29 SHV 2b enzymes have been described (G. Jacoby and K. Bush, <http://www.lahey.org/Studies/>) often in the course of characterizing other  $\beta$ -lactamases in unusually resistant clinical isolates.

Subgroup 2be comprises ESBLs. These broad-spectrum enzymes retain the activity against penicillins and cephalosporins of subgroup 2b  $\beta$ -lactamases and in addition hydrolyze one or more oximino- $\beta$ -lactams, such as cefotaxime, ceftazidime, and aztreonam, at a rate generally  $>10\%$  that of benzylpenicillin. The first and largest subset of subgroup 2be was derived by amino acid substitutions in TEM-1, TEM-2, and SHV-1 that broadened their substrate spectrum at a cost of lower hydrolyzing activity for benzylpenicillin and cephaloridine (53). TEM and SHV ESBLs have been joined by the functionally similar but more rapidly proliferating CTX-M enzymes that are related to chromosomally determined  $\beta$ -lactamases in species of *Kluyvera* (8). As the name implies, most (but not all) CTX-M enzymes hydrolyze cefotaxime more readily than ceftazidime. Many hydrolyze cefepime as well. Unlike TEM or SHV ESBLs, CTX-M enzymes are inhibited by tazobactam at least an order of magnitude better than by clavulanic acid (8, 65). Finally, there are less common ESBLs unrelated to TEM, SHV, or CTX-M, including BEL-1, BES-1, SFO-1, TLA-1, TLA-2, and members of the PER and VEB enzyme families. Characteristically, subgroup 2be  $\beta$ -lactamases remain sensitive to inhibition by clavulanic acid, a feature used in their detection by clinical laboratories (19).

Subgroup 2br enzymes are broad-spectrum  $\beta$ -lactamases that have acquired resistance to clavulanic acid ( $IC_{50} \geq 1 \mu\text{M}$ ) and related inhibitors while retaining a subgroup 2b spectrum of activity. Currently 36 of the 135 functionally characterized TEM enzymes have this property and include enzymes such as TEM-30 and TEM-31 (IRT-2 and IRT-1, respectively), as well as 5 of the corresponding functionally characterized 72 SHV enzymes (e.g., SHV-10). No CTX-M  $\beta$ -lactamase demonstrates this characteristic to date (G. Jacoby and K. Bush, <http://www.lahey.org/Studies/>).

Subgroup 2ber includes TEM enzymes that combine an extended spectrum with relative resistance to clavulanic acid inhibition. Although all have clavulanic acid  $IC_{50}$ s greater than that of TEM-1 ( $0.08 \mu\text{M}$ ), for some 2ber enzymes the increase in clavulanic acid resistance is modest. They have also been termed CMT (complex mutant TEM)  $\beta$ -lactamases and include TEM-50 (CMT-1) (56, 61).

Subgroup 2c penicillinases are characterized functionally by

their ability to hydrolyze carbenicillin or ticarcillin at least 60% as rapidly as benzylpenicillin, with cloxacillin or oxacillin hydrolyzed at rates less than half those for benzylpenicillin (16). These penicillinases are generally easily inhibited by clavulanic acid or tazobactam, most often with  $IC_{50}$ s of  $<1 \mu\text{M}$ . Because carbenicillin is an antibiotic that is currently used infrequently and is not tested for stability by most investigators, only a few new 2c  $\beta$ -lactamases have been described over the past decade (18, 40, 47).

Subgroup 2ce contains the recently described extended-spectrum carbenicillinase RTG-4 (CARB-10) with expanded activity against cefepime and ceftipime (50).

Subgroup 2d includes  $\beta$ -lactamases distinguished by their ability to hydrolyze cloxacillin or oxacillin at a rate of  $>50\%$  that for benzylpenicillin and hence are known as OXA enzymes. Carbenicillin may also be readily hydrolyzed. Most members of the OXA family, however, are currently identified according to their conserved amino acid motifs rather than according to function. Many  $\beta$ -lactamases in this subgroup are inhibited by NaCl; they typically have clavulanic acid  $IC_{50}$ s of  $\geq 1 \mu\text{M}$ . OXA-related enzymes now comprise the second largest family of  $\beta$ -lactamases (Table 2).

In the new subgroup 2de are cloxacillin- or oxacillin-hydrolyzing enzymes with an extended spectrum that includes oximino- $\beta$ -lactams but not carbapenems. The majority of 2de enzymes are derived from OXA-10 by between 1 and 9 amino acid substitutions and include enzymes such as OXA-11 and OXA-15. They have most often been found in Turkey and France in isolates of *P. aeruginosa*, where the level of resistance they produce is higher than that in *E. coli* (9). Resistance to ceftazidime is usually more pronounced than resistance to cefotaxime or aztreonam. However, organisms producing a few oxacillinases, such as OXA-1 or OXA-31, may be susceptible to ceftazidime but resistant to cefepime (4).

New subgroup 2df  $\beta$ -lactamases are OXA enzymes with carbapenem-hydrolyzing activities. They appear most frequently in *Acinetobacter baumannii* and are usually produced by genes that are located on the chromosome (66), although plasmid-borne OXA-23 and OXA-48 enzymes have been identified in the *Enterobacteriaceae* (16, 48). The 2df enzymes have been divided into nine clusters according to amino acid homologies (52, 59, 62, 66). Although subgroup 2d enzymes are defined functionally according to their ability to hydrolyze cloxacillin or oxacillin, only a few subgroup 2df enzymes have been tested using these substrates (66). Of those tested, only OXA-50 had no detectable oxacillin hydrolysis. The characterized OXA carbapenemases have weak hydrolytic activity for carbapenems, demonstrated by  $k_{\text{cat}}$  values for imipenem and meropenem that are generally  $\leq 1 \text{ s}^{-1}$ , with imipenem hydrolyzed faster and more efficiently than meropenem. These rates compare to much higher  $k_{\text{cat}}$  values for benzylpenicillin or oxacillin, substrates that were usually hydrolyzed at least 40- to 50-fold faster than the carbapenems (66). Although the producing organisms are generally highly resistant to carbapenems, *E. coli* transformants or transconjugants that produce these enzymes are usually susceptible to the carbapenems (66). The enzymes, and their producing organisms, are typically unresponsive to inhibition by clavulanic acid.

Characteristics of the subgroup 2e cephalosporinases include the ability to hydrolyze extended-spectrum cephalosporins

rins and to be inhibited by clavulanic acid or tazobactam. The inducible, chromosomal cephalosporinases in the *Proteae* often belong to this subgroup. They can be confused with the group 1 AmpC enzymes or with ESBLs because they may appear in similar organisms and with comparable resistance profiles. Subgroup 2e enzymes can be differentiated from AmpC enzymes by their poor affinity for aztreonam, in contrast to the nM  $K_m$  ( $K_i$ ) values observed for aztreonam with group 1 enzymes (13). The number of 2e enzymes has remained stable since 1995 and is not expected to include many new members in the future, probably because many of these enzymes are now identified as ESBLs.

Serine carbapenemases from molecular class A populate subgroup 2f. Carbapenems are the distinctive substrates for these enzymes, which can be inhibited better by tazobactam than by clavulanic acid. Extended-spectrum cephalosporins such as ceftazidime are not well hydrolyzed by the SME and IMI-1 enzymes, but aztreonam can be degraded by most of them, except for GES-3 and GES-4. The SME family, as well as IMI-1 and NMC-1  $\beta$ -lactamases, are representatives of the chromosomal subgroup 2f enzymes (52). More worrisome, however, are the plasmid-encoded subgroup 2f  $\beta$ -lactamases, including KPC and some GES (formerly IBC) enzymes. The KPC carbapenemases in particular have recently been associated with major outbreaks of multidrug-resistant Gram-negative infections in hospitals, including those in the New York City metropolitan area (10, 12, 70) and in Israel (32), with their spread now becoming worldwide (43, 63, 68).

**Group 3 MBLs.** Metallo- $\beta$ -lactamases (MBLs), a unique group of  $\beta$ -lactamases both structurally and functionally, are usually produced in combination with a second or third  $\beta$ -lactamase in clinical isolates. They differ structurally from the other  $\beta$ -lactamases by their requirement for a zinc ion at the active site. Functionally, they were once distinguished primarily by their ability to hydrolyze carbapenems, but some serine  $\beta$ -lactamases now have also acquired that ability. In contrast to the serine  $\beta$ -lactamases, the MBLs have poor affinity or hydrolytic capability for monobactams and are not inhibited by clavulanic acid or tazobactam. Instead, they are inhibited by metal ion chelators such as EDTA, dipicolinic acid, or 1,10-*o*-phenanthroline (31, 37). These metalloenzymes have been subdivided, based on either structure (subclasses B1, B2, and B3) (22–24) or function (subgroups 3a, 3b, and 3c) (54). As with the other functional groups, the two groupings were aligned as closely as possible, although structural subclasses B1 and B3 were found to correlate with similar functions (Table 1). MBLs originally were identified as chromosomal enzymes in Gram-positive or occasional Gram-negative bacilli, such as *Bacteroides fragilis* (72) or *Stenotrophomonas maltophilia* (35, 42), and their number accordingly remained relatively constant for many years. When MBLs began to appear on transferable elements, they became more promiscuous and were subject to evolutionary pressures in a variety of hosts, resulting in enzyme families with several dozen unique variants (Table 2).

Based on more extensive biochemical characterization of the increasing numbers of metallo- $\beta$ -lactamases, it is now being proposed that only two functional subgroups be described. Subgroup 3a includes the major plasmid-encoded MBL families, such as the IMP and VIM enzymes that have appeared globally, most frequently in nonfermentative bacteria but also

in *Enterobacteriaceae* (52). These enzymes belong to molecular subclass B1 based on the consensus amino acids that serve as ligands to the two zinc atoms required for the broad-spectrum hydrolytic activity observed with these MBLs (22–24). In addition, the common L1 MBL from *S. maltophilia* as well as the subclass B3 MBLs, such as CAU-1, GOB-1, and FEZ-1, are being added to subgroup 3a (7, 54). These enzymes differ from the other subgroup 3a enzymes due to differences in the amino acids involved in zinc binding; however, both structural subclasses require two bound zinc ions for maximal enzymatic activity and have similar broad-spectrum substrate profiles (23, 24). High hydrolysis rates based both on  $k_{cat}$  and  $k_{cat}/K_m$  values are observed for penicillins, cephalosporins, and carbapenems, but not monobactams. An exception is the FEZ-1 carbapenemase with preferential hydrolysis of cephalosporins compared to carbapenems and penicillins, due primarily to high  $K_m$  values for these latter substrates (41).

Subgroup 3b contains a smaller group of MBLs that preferentially hydrolyze carbapenems in contrast to penicillins and cephalosporins (60). These enzymes have been difficult to detect when chromogenic cephalosporins, such as nitrocefin, are used to monitor the presence of  $\beta$ -lactamase activity on isoelectric focusing gels or during purification procedures. Thus, the chromosomal MBLs in *Aeromonas* spp. were often overlooked in carbapenem-resistant isolates because the enzymes did not react with nitrocefin in cell extracts used for isoelectric focusing or during chromatography (71). Mechanistically, these enzymes are most effective in hydrolyzing carbapenems if only one of the zinc binding sites is occupied (26). In contrast to the other subgroups of MBLs, the presence of a second zinc ion is actually inhibitory to enzymatic activity (20).

Group 4  $\beta$ -lactamases previously included in the 1995 functional classification have been omitted in the present scheme. These enzymes most likely would be included in one of the existing enzyme groups if more information about them were available. Because these enzymes have as yet been incompletely characterized, further categorization has not been attempted.

## DISCUSSION

Ideally, a strong structure-function relationship should be observed among the various  $\beta$ -lactamase groupings. The optimal outcome would result in a categorization scheme that placed all  $\beta$ -lactamases into a single classification grid aligning both structure and function. This kind of relationship is beginning to be accomplished with the MBLs. However, at the current time, many  $\beta$ -lactamases are described only on the basis of a protein sequence, with little functional description. Therefore, a set of criteria has been proposed for the description of a new  $\beta$ -lactamase, including both structural and functional information. Table 3 outlines the information needed for a full characterization.

The reasons for  $\beta$ -lactamase diversity are many. At least the serine-based varieties are ancient enzymes, estimated to have been evolving for more than 2 billion years starting from a time before the divergence of bacteria into Gram-negative and Gram-positive varieties (25). They are found in bacteria living in a wide variety of environments and hence are subject to different selective pressures. They are well-studied enzymes

TABLE 3. Characterization of new  $\beta$ -lactamases

Data set required	Specific testing criteria
MICs using standardized methodology .....	Select antibiotics that most closely define the enzymatic properties. Strains to be tested should include (i) original clinical isolate, (ii) transformant or transconjugant with no other enzyme present, and (iii) host strain without any $\beta$ -lactamase.
Full nucleotide and amino acid sequences .....	Standard methodology
Approved name .....	Contact the curators of the website <a href="http://www.lahey.org/Studies/">http://www.lahey.org/Studies/</a>
Protein purification .....	At least 90% purity on electrophoresis with high protein concn. No unrelated $\beta$ -lactamase activity in the extract.
Isoelectric focusing .....	Isoelectric point for all $\beta$ -lactamases in original clinical isolate and for purified protein
Hydrolysis profile ( $k_{\text{cat}}$ and $K_m$ values) .....	Standard substrates are as follows: cephaloridine <sup>a</sup> (or cephalothin), benzylpenicillin, cloxacillin or oxacillin, cefotaxime, ceftazidime, cefoxitin, imipenem, and aztreonam. Additional substrates should be tested for enzymes with specific substrate profiles, e.g., doripenem, ertapenem, and/or meropenem for carbapenemases. Native (nontagged) enzymes should be studied.
Inhibition profile ( $\text{IC}_{50}$ <sup>b</sup> or inhibition constants) .....	Standard inhibitors, including clavulanic acid, tazobactam, and EDTA <sup>c</sup>

<sup>a</sup> Cephaloridine is preferred (contact K. Bush for availability), but cephalothin can be substituted.

<sup>b</sup> Determined after 5 min of preincubation of enzyme and inhibitor. Substrate concentration and  $K_m$  should be reported.

<sup>c</sup> Other metal ion-chelating agents, such as 1,10-*o*-phenanthroline or dipicolinic acid, can also be tested if an MBL is suspected.

that have attracted the attention of many investigators in the 70 years since they were first described. They are adaptable enzymes that have evolved to avoid being crippled by compounds intended as inhibitors and to attack  $\beta$ -lactam antibiotics designed to resist their action (39). Finally, *bla* genes have profited from the many mechanisms for horizontal gene transfer between bacteria to spread to new hosts and to become part of multiresistance plasmids now common in clinical isolates with resulting promiscuous dissemination. Given these many factors, it is a safe prediction that  $\beta$ -lactamases will continue to evolve, as will classification schemes needed for their description.

#### ACKNOWLEDGMENTS

We thank all of the  $\beta$ -lactamase investigators who have contributed to the extensive literature on these complex and varied enzymes. We especially thank our collaborator Antone Medeiros, who was a major contributor to the 1995 functional classification.

#### REFERENCES

- Ahmed, A. M., and T. Shimamoto. 2008. Emergence of a cefepime- and ceftipime-resistant *Citrobacter freundii* clinical isolate harbouring a novel chromosomally encoded AmpC  $\beta$ -lactamase, CMY-37. *Int. J. Antimicrob. Agents* **32**:256–261.
- Ambler, R. P. 1980. The structure of  $\beta$ -lactamases. *Philos. Trans. R. Soc. Lond. B* **289**:321–331.
- Ambler, R. P., A. F. W. Coulson, J.-M. Frère, J.-M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley. 1991. A standard numbering scheme for the class A  $\beta$ -lactamases. *Biochem. J.* **276**:269–272.
- Aubert, D., L. Poirel, J. Chevalier, S. Leotard, J. M. Pages, and P. Nordmann. 2001. Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **45**:1615–1620.
- Babini, G. S., F. Danel, S. D. Munro, P. A. Micklesen, and D. M. Livermore. 1998. Unusual tazobactam-sensitive AmpC  $\beta$ -lactamase from two *Escherichia coli* isolates. *J. Antimicrob. Chemother.* **41**:115–118.
- Bauernfeind, A., I. Schneider, R. Jungwirth, H. Sahly, and U. Ullmann. 1999. A novel type of AmpC  $\beta$ -lactamase, ACC-1, produced by a *Klebsiella pneumoniae* strain causing nosocomial pneumonia. *Antimicrob. Agents Chemother.* **43**:1924–1931.
- Bellais, S., D. Aubert, T. Naas, and P. Nordmann. 2000. Molecular and biochemical heterogeneity of class B carbapenem-hydrolyzing  $\beta$ -lactamases in *Chryseobacterium meningosepticum*. *Antimicrob. Agents Chemother.* **44**:1878–1886.
- Bonnet, R. 2004. Growing group of extended-spectrum  $\beta$ -lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother.* **48**:1–14.
- Bradford, P. A. 2001. Extended-spectrum  $\beta$ -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* **14**:933–951.
- Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale. 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30  $\beta$ -lactamases in New York City. *Clin. Infect. Dis.* **39**:55–60.
- Bradford, P. A., C. Urban, N. Mariano, S. J. Projan, J. J. Rahal, and K. Bush. 1997. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC  $\beta$ -lactamase, and the loss of an outer membrane protein. *Antimicrob. Agents Chemother.* **41**:563–569.
- Bratu, S., D. Landman, R. Haag, R. Recco, A. Eramo, M. Alam, and J. Quale. 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. *Arch. Intern. Med.* **165**:1430–1435.
- Bush, K. 1989. Characterization of  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* **33**:259–263.
- Bush, K. 1988.  $\beta$ -Lactamase inhibitors from laboratory to clinic. *Clin. Microbiol. Rev.* **1**:109–123.
- Bush, K., J. S. Freudenberger, and R. B. Sykes. 1982. Interaction of azthreonam and related monobactams with  $\beta$ -lactamases from gram-negative bacteria. *Antimicrob. Agents Chemother.* **22**:414–420.
- Bush, K., G. A. Jacoby, and A. A. Medeiros. 1995. A functional classification scheme for  $\beta$ -lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother.* **39**:1211–1233.
- Bush, K., S. K. Tanaka, D. P. Bonner, and R. B. Sykes. 1985. Resistance caused by decreased penetration of  $\beta$ -lactam antibiotics into *Enterobacter cloacae*. *Antimicrob. Agents Chemother.* **27**:555–560.
- Choury, D., M. F. Szajnert, M. L. Joly-Guillou, K. Azibi, M. Delpech, and G. Paul. 2000. Nucleotide sequence of the *bla*<sub>RTG-2</sub> (CARB-5) gene and phylogeny of a new group of carbenicillinases. *Antimicrob. Agents Chemother.* **44**:1070–1074.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
- Costello, A. L., N. P. Sharma, K. W. Yang, M. W. Crowder, and D. L. Tierney. 2006. X-ray absorption spectroscopy of the zinc-binding sites in the class B2 metallo- $\beta$ -lactamase ImiS from *Aeromonas veronii* bv. *sobria*. *Biochemistry* **45**:13650–13658.
- Doi, Y., D. L. Paterson, J. M. Adams-Haduch, H. E. Sidjabat, A. O'Keefe, A. Endimiani, and R. A. Bonomo. 2009. Reduced susceptibility to cefepime among *Escherichia coli* clinical isolates producing novel variants of CMY-2  $\beta$ -lactamase. *Antimicrob. Agents Chemother.* **53**:3159–3161.
- Frère, J. M., M. Galleni, K. Bush, and O. Dideberg. 2005. Is it necessary to change the classification of  $\beta$ -lactamases? *J. Antimicrob. Chemother.* **55**:1051–1053.
- Galleni, M., J. Lamotte-Brasseur, G. M. Rossolini, J. Spencer, O. Dideberg, and J. M. Frère. 2001. Standard numbering scheme for class B  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* **45**:660–663.
- Garau, G., I. García-Sáez, C. Bebrone, C. Anne, P. Mercuri, M. Galleni, J. M. Frère, and O. Dideberg. 2004. Update of the standard numbering

- scheme for class B  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* **48**:2347–2349.
25. Hall, B. G., and M. Barlow. 2003. Structure-based phylogenies of the serine  $\beta$ -lactamases. *J. Mol. Evol.* **57**:255–260.
  26. Hernandez Valladares, M., A. Felici, G. Weber, H. W. Adolph, M. Zeppeauer, G. M. Rossolini, G. Amicosante, J. M. Frère, and M. Galleni. 1997. Zn(II) dependence of the *Aeromonas hydrophila* AE036 metallo- $\beta$ -lactamase activity and stability. *Biochemistry* **36**:11534–11541.
  27. Jacoby, G. A. 2009. AmpC  $\beta$ -lactamases. *Clin. Microbiol. Rev.* **22**:161–182.
  28. Jacoby, G. A., D. M. Mills, and N. Chow. 2004. Role of  $\beta$ -lactamases and porins in resistance to ertapenem and other  $\beta$ -lactams in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **48**:3203–3206.
  29. Jaurin, B., and T. Grundstrom. 1981. *ampC* cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of  $\beta$ -lactamases of the penicillinase type. *Proc. Natl. Acad. Sci. U. S. A.* **78**:4897–4901.
  30. Kernodle, D. S., C. W. Stratton, L. W. McMurray, J. R. Chipley, and P. A. McGraw. 1989. Differentiation of  $\beta$ -lactamase variants of *Staphylococcus aureus* by substrate hydrolysis profiles. *J. Infect. Dis.* **159**:103–108.
  31. Laraki, N., N. Franceschini, G. M. Rossolini, P. Santucci, C. Meunier, E. de Pauw, G. Amicosante, J. M. Frère, and M. Galleni. 1999. Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo- $\beta$ -lactamase IMP-1 produced by *Escherichia coli*. *Antimicrob. Agents Chemother.* **43**:902–906.
  32. Leavitt, A., S. Navon-Venezia, I. Chmelnitsky, M. J. Schwaber, and Y. Carmeli. 2007. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. *Antimicrob. Agents Chemother.* **51**:3026–3029.
  33. Lee, S. H., S. H. Jeong, and Y. M. Park. 2003. Characterization of *bla*<sub>CMY-10</sub> a novel, plasmid-encoded AmpC-type  $\beta$ -lactamase gene in a clinical isolate of *Enterobacter aerogenes*. *J. Appl. Microbiol.* **95**:744–752.
  34. Livermore, D. M. 1987. Clinical significance of beta-lactamase induction and stable derepression in gram-negative rods. *Eur. J. Clin. Microbiol.* **6**:439–445.
  35. Livermore, D. M., and N. Woodford. 2000. Carbapenemases: a problem in waiting? *Curr. Opin. Microbiol.* **3**:489–495.
  36. Mammeri, H., P. Nordmann, A. Berkani, and F. Eb. 2008. Contribution of extended-spectrum AmpC (ESAC)  $\beta$ -lactamases to carbapenem resistance in *Escherichia coli*. *FEMS Microbiol. Lett.* **282**:238–240.
  37. Marchiaro, P., V. Ballerini, T. Spalding, G. Cera, M. A. Mussi, J. Moran-Barrio, A. J. Vila, A. M. Viale, and A. S. Limansky. 2008. A convenient microbiological assay employing cell-free extracts for the rapid characterization of Gram-negative carbapenemase producers. *J. Antimicrob. Chemother.* **62**:336–344.
  38. Matthew, M. 1979. Plasmid mediated  $\beta$ -lactamases of gram-negative bacteria: properties and distribution. *J. Antimicrob. Chemother.* **5**:349–358.
  39. Medeiros, A. A. 1997. Evolution and dissemination of  $\beta$ -lactamases accelerated by generations of  $\beta$ -lactam antibiotics. *Clin. Infect. Dis.* **24**(Suppl. 1):S19–S45.
  40. Melano, R., A. Petroni, A. Garutti, H. A. Saka, L. Mange, F. Pasteran, M. Rapoport, A. Rossi, and M. Galas. 2002. New carbenicillin-hydrolyzing  $\beta$ -lactamase (CARB-7) from *Vibrio cholerae* non-O1, non-O139 strains encoded by the VCR region of the *V. cholerae* genome. *Antimicrob. Agents Chemother.* **46**:2162–2168.
  41. Mercuri, P. S., F. Bouillenne, L. Boschi, J. Lamotte-Brasseur, G. Amicosante, B. Devreese, J. van Beunem, J. M. Frere, G. M. Rossolini, and M. Galleni. 2001. Biochemical characterization of the FEZ-1 metallo- $\beta$ -lactamase of *Legionella gormanii* ATCC 33297<sup>T</sup> produced in *Escherichia coli*. *Antimicrob. Agents Chemother.* **45**:1254–1262.
  42. Mercuri, P. S., Y. Ishii, L. Ma, G. M. Rossolini, F. Luzzaro, G. Amicosante, N. Franceschini, J. M. Frère, and M. Galleni. 2002. Clonal diversity and metallo- $\beta$ -lactamase production in clinical isolates of *Stenotrophomonas maltophilia*. *Microb. Drug Resist.* **8**:193–200.
  43. Naas, T., P. Nordmann, G. Vedel, and C. Poyart. 2005. Plasmid-mediated carbapenem-hydrolyzing  $\beta$ -lactamase KPC in a *Klebsiella pneumoniae* isolate from France. *Antimicrob. Agents Chemother.* **49**:4423–4424.
  44. Nordmann, P., and H. Mammeri. 2007. Extended-spectrum cephalosporinases: structure, detection and epidemiology. *Future Microbiol.* **2**:297–307.
  45. Nukaga, M., S. Haruta, K. Tanimoto, K. Kogure, K. Taniguchi, M. Tamaki, and T. Sawai. 1995. Molecular evolution of a class C  $\beta$ -lactamase extending its substrate specificity. *J. Biol. Chem.* **270**:5729–5735.
  46. Ouellette, M., L. Bissonnette, and P. H. Roy. 1987. Precise insertion of antibiotic resistance determinants into Tn21-like transposons: nucleotide sequence of the OXA-1 beta-lactamase gene. *Proc. Natl. Acad. Sci. U. S. A.* **84**:7378–7382.
  47. Petroni, A., R. G. Melano, H. A. Saka, A. Garutti, L. Mange, F. Pasteran, M. Rapoport, M. Miranda, D. Faccione, A. Rossi, P. S. Hoffman, and M. F. Galas. 2004. CARB-9, a carbenicillinase encoded in the VCR region of *Vibrio cholerae* non-O1, non-O139 belongs to a family of cassette-encoded  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* **48**:4042–4046.
  48. Poirel, L., C. Heritier, V. Tolun, and P. Nordmann. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **48**:15–22.
  49. Poirel, L., G. F. Weldhagen, T. Naas, C. De Champs, M. G. Dove, and P. Nordmann. 2001. GES-2, a class A  $\beta$ -lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. *Antimicrob. Agents Chemother.* **45**:2598–2603.
  50. Potron, A., L. Poirel, J. Croizé, V. Chantepredrix, and P. Nordmann. 2009. Genetic and biochemical characterization of the first extended-spectrum CARB-type  $\beta$ -lactamase, RTG-4, from *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **53**:3010–3016.
  51. Quale, J., S. Bratu, J. Gupta, and D. Landman. 2006. Interplay of efflux system, ampC, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob. Agents Chemother.* **50**:1633–1641.
  52. Queenan, A. M., and K. Bush. 2007. Carbapenemases: the versatile  $\beta$ -lactamases. *Clin. Microbiol. Rev.* **20**:440–458.
  53. Queenan, A. M., B. Foleo, C. Gownley, E. Wira, and K. Bush. 2004. Effects of inoculum and  $\beta$ -lactamase activity in AmpC- and extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. *J. Clin. Microbiol.* **42**:269–275.
  54. Rasmussen, B. A., and K. Bush. 1997. Carbapenem-hydrolyzing  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* **41**:223–232.
  55. Richmond, M. H., and R. B. Sykes. 1973. The  $\beta$ -lactamases of gram-negative bacteria and their possible physiological roles. *Adv. Microb. Physiol.* **9**:31–88.
  56. Robin, F., J. Delmas, C. Chanal, D. Siro, J. Siro, and R. Bonnet. 2005. TEM-109 (CMT-5), a natural complex mutant of TEM-1  $\beta$ -lactamase combining the amino acid substitutions of TEM-6 and TEM-33 (IRT-5). *Antimicrob. Agents Chemother.* **49**:4443–4447.
  57. Rodríguez-Martínez, J. M., L. Poirel, and P. Nordmann. 2009. Extended-spectrum cephalosporinases in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **53**:1766–1771.
  58. Roy, C., A. Foz, C. Segura, M. Tirado, C. Fuster, and R. Reig. 1983. Plasmid-determined  $\beta$ -lactamases identified in a group of 204 ampicillin-resistant *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **12**:507–510.
  59. Schneider, I., A. M. Queenan, and A. Bauernfeind. 2006. Novel carbapenem-hydrolyzing oxacillinase OXA-62 from *Pandora phenomena*. *Antimicrob. Agents Chemother.* **50**:1330–1335.
  60. Segatore, B., O. Massidda, G. Satta, D. Setacci, and G. Amicosante. 1993. High specificity of *cphA*-encoded metallo- $\beta$ -lactamase from *Aeromonas hydrophila* AE036 for carbapenems and its contribution to  $\beta$ -lactam resistance. *Antimicrob. Agents Chemother.* **37**:1324–1328.
  61. Siro, D., C. Recule, E. B. Chaibi, L. Bret, J. Croize, C. Chanal-Claris, R. Labia, and J. Siro. 1997. A complex mutant of TEM-1  $\beta$ -lactamase with mutations encountered in both IRT-4 and extended-spectrum TEM-15, produced by an *Escherichia coli* clinical isolate. *Antimicrob. Agents Chemother.* **41**:1322–1325.
  62. Turton, J. F., M. E. Ward, N. Woodford, M. E. Kaufmann, R. Pike, D. M. Livermore, and T. L. Pitt. 2006. The role of ISAbal in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol. Lett.* **258**:72–77.
  63. Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, and J. P. Quinn. 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing  $\beta$ -lactamase. *Antimicrob. Agents Chemother.* **51**:1553–1555.
  64. Wachino, J., H. Kurokawa, S. Suzuki, K. Yamane, N. Shibata, K. Kimura, Y. Ike, and Y. Arakawa. 2006. Horizontal transfer of *bla*<sub>CMY</sub>-bearing plasmids among clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates and emergence of cefepime-hydrolyzing CMY-19. *Antimicrob. Agents Chemother.* **50**:534–541.
  65. Walther-Rasmussen, J., and N. Hoiby. 2004. Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum  $\beta$ -lactamases. *Can. J. Microbiol.* **50**:137–165.
  66. Walther-Rasmussen, J., and N. Hoiby. 2006. OXA-type carbapenemases. *J. Antimicrob. Chemother.* **57**:373–383.
  67. Weber, D. A., and C. C. Sanders. 1990. Diverse potential of  $\beta$ -lactamase inhibitors to induce class I enzymes. *Antimicrob. Agents Chemother.* **34**:156–158.
  68. Wei, Z. Q., X. X. Du, Y. S. Yu, P. Shen, Y. G. Chen, and L. J. Li. 2007. Plasmid-mediated KPC-2 in a *Klebsiella pneumoniae* isolate from China. *Antimicrob. Agents Chemother.* **51**:763–765.
  69. Wong-Beringer, A., J. Hindler, M. Loeloff, A. M. Queenan, N. Lee, D. A. Pegues, J. P. Quinn, and K. Bush. 2002. Molecular correlation for the treatment outcomes in bloodstream infections caused by *Escherichia coli* and *Klebsiella pneumoniae* with reduced susceptibility to ceftazidime. *Clin. Infect. Dis.* **34**:135–146.
  70. Woodford, N., P. M. Tierno, Jr., K. Young, L. Tysall, M. F. Palepou, E. Ward, R. E. Painter, D. F. Suber, D. Shungu, L. L. Silver, K. Inglima, J. Kornblum, and D. M. Livermore. 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A  $\beta$ -lactamase, KPC-3, in a New York Medical Center. *Antimicrob. Agents Chemother.* **48**:4793–4799.
  71. Yang, Y., and K. Bush. 1996. Biochemical characterization of the carbapenem-hydrolyzing  $\beta$ -lactamase AsbM1 from *Aeromonas sobria* AER 14M: a

- member of a novel subgroup of metallo- $\beta$ -lactamases. FEMS Microbiol. Lett. **137**:193–200.
72. Yotsuji, A., S. Minami, M. Inoue, and S. Mitsuhashi. 1983. Properties of novel  $\beta$ -lactamase produced by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **24**:925–929.
73. Yu, W. L., W. C. Ko, K. C. Cheng, H. E. Chen, C. C. Lee, and Y. C. Chuang. 2008. Institutional spread of clonally related *Serratia marcescens* isolates with a novel AmpC cephalosporinase (S4): a 4-year experience in Taiwan. Diagn. Microbiol. Infect. Dis. **61**:460–467.
74. Zscheck, K. K., and B. E. Murray. 1991. Nucleotide sequence of the  $\beta$ -lactamase gene from *Enterococcus faecalis* HH22 and its similarity to staphylococcal  $\beta$ -lactamase genes. Antimicrob. Agents Chemother. **35**:1736–1740.

**Karen Bush** received her B.A. from Monmouth College (IL) and her Ph.D. in Biochemistry from Indiana University. She was involved in antibiotic Discovery and Development programs at E. R. Squibb & Sons, Bristol-Myers Squibb, American Cyanamid/Lederle/Wyeth, Astra, and, most recently, Johnson & Johnson Pharmaceutical Research & Development, where she was a Distinguished Research Fellow and the Microbiology Team Leader in Preclinical Anti-Infective Research. She is semiretired and is currently an Adjunct Professor of Biology at Indiana University Bloomington. Her primary interests have been related to antiinfective drug discovery based on attempts to counteract antibiotic resistance, particularly with respect to  $\beta$ -lactams. She began studying  $\beta$ -lactamases in 1977 and still retains an active interest in tracking the new enzymes that continue to be described.



**George A. Jacoby** trained at Harvard Medical School, the National Institutes of Health, the National Institute for Medical Research at Mill Hill, and Massachusetts General Hospital, where he was a consultant in the Infectious Disease Unit for 25 years before moving in 1993 to head the Infectious Disease Department at the Lahey Clinic. He retired from clinical work in 2002 and now heads a research lab at Lahey, where he works on bacterial resistance to antimicrobial agents, especially quinolones and  $\beta$ -lactams. He is an Associate Professor of Medicine at Harvard Medical School and began working with  $\beta$ -lactamases in 1963.

